

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Masakazu TAKEUCHI, et al.

Application No.: 10/536,586

Filed: November 28, 2003

For: MAMMALIAN PRICKLE GENE

Customer No.: 20350

Confirmation No. 9187

Examiner: Chernyshev, Olga N.

Technology Center/Art Unit: 1649

DECLARATION UNDER 37 C.F.R. §1.132
OF DR. MASAKAZU TAKEUCHI, PH.D.

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Dr. Masakazu Takeuchi, being duly warned that willful false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements made herein of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

2. I am currently working the Planning & Coordination Department of KAN Research Institute, Inc. At the time of the present invention, I was a Principal Investigator in the Neurobiology Group at KAN Research Institute. I have studied neurobiology for over 10 years and have published over 20 articles in this field. Furthermore, I have obtained three patents in this field. A copy of my CV is attached (**Exhibit A**).

3. I am the named inventor on this patent application. I have read and am familiar with the contents of this patent application. In addition, I have read the Final Office Action mailed April 10, 2008, for the present application. It is my understanding that the Examiner has rejected the pending claims, *inter alia*, for alleged lack of utility.

4. The claimed gene and the protein encoded by the gene, or fragments thereof, can be used, *inter alia*, to screen for compounds that modulate the post-synaptic density, and thereby affect the physiological processes underlying learning and memory. Thus, as described in more detail below, the nucleic acid and proteins or polypeptides encoded by the mammalian prickly gene can be used to screen for compounds that affect learning and memory.

5. At the time of the invention, it was well known that postsynaptic density (PSD) contributes to information processing and the formation of memories by changing synaptic strength in response to neural activity. *See, Exhibit B, Kennedy et al. Science 290:750-754 page 750 abstract lines 9-11.* At the time of the invention, a skilled artisan would readily conclude that proteins associated with the PSD contribute to memory formation. Notably, the inventors have determined that the m-Prickle protein is concentrated in the PSD fraction (see Example 6, and Figure 5 of the specification). Thus, based on this observation, one of skill would conclude that the m-Prickle protein contributes to memory formation.

6. Furthermore, it was also known that PSD-95 at synapses plays a functional role in learning and memory by interacting with other signal molecules. In fact, as described in Migaud *et al.* (Submitted with the response in the response filed on January 30, 2008), mutant mice that lacked functional PSD-95 showed a marked inability to learn the position of a hidden platform (page 437, right column, lines 39-41). It was also well known that PSD-95 forms a complex with the NMDA receptors (NMDA-R), which are known to play a pivotal role in memory formation and learning (*see, e.g. Migaud, M. et al. abstract and figure 6, page 438*). Thus, PSD-95 was known to play a functional role in learning.

7. The present invention also shows that the m-Prickle protein possesses LIM domains, which are known to function as protein interaction domains, mediating specific contacts between members of functional complexes and modulating the activity of constituent proteins (*see, e.g., abstract of Dawid I.B., et al. (1998) Trends in Genet. 14:156-162, submitted with the response in the response filed on January 30, 2008*). Based on this observation, one of skill would recognize that the m-Prickle protein plays a role in learning and memory by interacting with PSD-95 via its LIM domains.

8. Even more importantly, the inventors have shown that the m-Prickle protein actually binds to the PSD-95 scaffold complex in the PSD (*see*, page 2, lines 20-21 and page 3, lines 20-22 of the specification). Indeed, the interaction of m-Prickle with the NMDA-R is evidenced by the present inventor's demonstration of the co-precipitation of m-Prickle with the NMDA-R using an anti-m-Prickle antibody.

9. In summary, based on understanding in the field at the time of the invention, one of skill would recognize that a protein that is concentrated in the PSD fraction and contains LIM domains known to be associated with protein interaction, such as m-Prickle is an adaptor molecule involved in learning and memory. This combined with evidence that m-Prickle actually binds the PSD-95 scaffold complex, establishes that the m-Prickle protein would be a good target for screening for compounds that affect learning and memory.

10. Declarant has nothing further to say.

Date: 4 August 2008

By: Masakazu Takeuchi

Attachments: Exhibits A-B

RCB:rcb
61418664 v1

EXHIBIT A

Masakazu Takeuchi Ph. D.

Planning & Coordination Department

KAN Research Institute, Inc.

3F, Kobe MI R&D Center

6-7-3 Minatojima-minamimachi, Chuo-ku, Kobe 650-0047, Japan

TEL. +81-78-306-5910

FAX. +81-78-306-5920

E-mail m-takeuchi@kan.eisai.co.jp

Education

April 1986-March 1990	Faculty of Science, Kyoto University (BS in Biochemistry)
April 1990-March 1992	Graduate School of Science, The University of Tokyo (MS in Biochemistry)
Nov 1999	Ph. D. in Neurobiology at Graduate School of Medicine, Osaka University

Employment

April 1992-July 1999	Researcher, Shionogi & Co., Ltd. (Osaka, Japan) Basic research in bone biology, neurobiology.
October 1999-March 2003	Principal investigator, Kan Research Institute, Inc. Neurobiology & histology
April 2003-present	Planning & Coordination Department, Kan Research Institute, Inc. Support for patent application, ensure research-legal matter.

Patents (US)

1. **7279461B2** (Oct. 9, 2007) Masakazu Takeuchi, Yoshimi Takai
Rabconnectin-3-binding protein
2. **7153831B2** (Dec. 26, 2006) Masakazu Takeuchi, Yoshimi Takai
Rabconnectin-3-binding protein
3. **7129063B2** (Oct. 31, 2006) Miyuki Nishimura, Mayumi Asano, Yuichi Ono, Koji Morimoto, Masakazu Takeuchi, Yoko Inoue, Toshio Imai, Yoshimi Takai
Exocrine gland tight junction-constituting protein jeep family

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EXHIBIT B

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98. We thank N. Spruston, C. Koch, C. Meunier, and M. Häusser for their constructive comments on this manuscript. The U.S.–Israel Binational Science Foundation, the Israeli Science Foundation, and the U.S. Office of Naval Research supported this work.

REVIEW

Signal-Processing Machines at the Postsynaptic Density

Mary B. Kennedy

Dendrites of individual neurons in the vertebrate central nervous system are contacted by thousands of synaptic terminals relaying information about the environment. The postsynaptic membrane at each synaptic terminal is the first place where information is processed as it converges on the dendrite. At the postsynaptic membrane of excitatory synapses, neurotransmitter receptors are attached to large protein "signaling machines" that delicately regulate the strength of synaptic transmission. These machines are visible in the electron microscope and are called the postsynaptic density. By changing synaptic strength in response to neural activity, the postsynaptic density contributes to information processing and the formation of memories.

Dendrites are the principal signal reception and processing sites on vertebrate neurons. The dendrites of each pyramidal neuron are highly branched and contain thousands of synapses made by axons from almost as many neurons. Most of these synapses are located on spines, which are tiny tubular or mushroom-shaped structures about 1 to 3 μm long and less than 1 μm in diameter that protrude from the dendritic shaft (Fig. 1). The typical presynaptic terminal forms a junction

with one, or at most two, postsynaptic spines. Spines are the first processing point for synaptic signals impinging on the dendrite. Much of the processing machinery is contained in a highly organized biochemical apparatus attached to the cytosolic surface of the postsynaptic membrane. This protein complex is visible in the electron microscope as a thickening of the postsynaptic membrane, extending approximately 30 nm into the cytosol; it was termed the "postsynaptic density" or PSD by early electron microscopists (Fig. 1) (1, 2).

Nearly all presynaptic terminals that make synapses on dendritic spines release the excita-

tory neurotransmitter glutamate. The postsynaptic membrane of a typical spine contains at least two distinct types of glutamate receptors concentrated at the site of contact with the presynaptic terminal. α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)-type glutamate receptors are ion channels that open when they bind glutamate, allowing sodium and potassium ions to flow across the membrane, producing a small, brief depolarization called the excitatory postsynaptic potential (EPSP). *N*-methyl-D-aspartate (NMDA)-type glutamate receptors are also ligand-gated ion channels. However, opening of their larger channel does not occur when glutamate binds to it, unless the membrane is strongly depolarized to relieve blockade of the channel by extracellular magnesium. The required depolarization is larger than can be achieved by AMPA receptors at a single synapse. Adequate depolarization can, in theory, be produced by coincident firing of several nearby synapses or by a back-propagating action potential (3). When the two conditions of glutamate binding and strong depolarization are met, the NMDA receptor channel opens and allows the flow of sodium and calcium ions into the cell. The resulting

Division of Biology, California Institute of Technology, Pasadena, CA 91125, USA. E-mail: kennedym@its.caltech.edu

influx of calcium ions is a powerful trigger that initiates a series of biochemical changes in the spine and neighboring dendrite. The result of these biochemical events is often a change in the size of the depolarization produced by AMPA receptors after a single electrical activation of the presynaptic terminal. Such changes in synaptic "strength" are believed to be an important mechanism by which information about past experiences is encoded at synapses. For example, by this mechanism, neurons that are activated at the same time during an experience might be "wired up" so that reactivation of one is more likely to activate all of them when the information is recalled.

The processes of thinking and of storing information in the brain are subtle and complex. Their accuracy is vital for the survival of the organism. Therefore, it is not surprising that the size and nature of the changes in synaptic strength produced by prior activation of a synapse are precisely regulated. The complexity of this regulation has often caused confusion, because nearly every known signaling pathway appears to play a role in synaptic plasticity in the central nervous system under some circumstance (4). Furthermore, the different signaling pathways can alter the strength of the synapse in several ways. Thus, the end result of the biochemical computation unleashed by the activation of NMDA receptors can be a change in the probability of transmitter release from an electrically activated presynaptic terminal (5), a change in the number of AMPA receptors (6, 7) or in the size of the current produced by each AMPA receptor at a postsynaptic site (8, 9), a change in the electrical excitability of the dendritic membrane (10), and/or coordinated changes in the cytoskeleton and in membrane trafficking that ultimately produce a new spine (11, 12). It seems likely that this profusion of mechanisms has evolved to permit the organism to store memories (or erase them), being guided by a wide variety of environmental influences.

The first potential targets of calcium ions entering through the NMDA receptor are proteins located in the PSD. One way to gain an understanding of postsynaptic signal processing is to unravel the structures and physical interconnections of the signaling proteins located in the PSD and then find ways to "watch" them in action. The protein structure of the PSD fraction, a portion of the PSD that remains intact upon homogenization of the brain and can be isolated by subcellular fractionation (13), reveals that signaling proteins at the postsynaptic membrane, like those in many other parts of the cell, are assembled in highly ordered arrays attached to the cytoplasmic surface (14–16). These "protein machines" appear to be built to direct signaling reactions in time and space and to incorporate mechanisms for modulating signaling at several intermediate steps.

The full complement of postsynaptic signaling proteins and their arrangement is likely to vary among different glutamatergic synapses. However, a model is emerging for the arrangement and interactions of the most prominent proteins in the PSD fraction. The abundance of these proteins suggests that they are likely to be present in a large percentage of glutamatergic PSDs. This initial model is based mostly on biochemical studies of proteins in the PSD fraction and of their points of interaction, defined by yeast two-hybrid experiments. Pharmacological and genetic studies hint at additional signaling mechanisms that remain to be precisely defined at the structural level.

The NMDA Receptor Signaling Complex

Many of the prominent proteins in the PSD fraction bind directly or indirectly to the NMDA receptor, which is itself an abundant component of the fraction. Thus, the PSD fraction contains a core NMDA receptor signaling complex. The NMDA receptor is composed of heteromultimers of four or five transmembrane subunits that include at least one NR1 subunit and an unknown number of NR2 subunits (NR2A, -B, -C, or -D) (17). The sequences of NR1 and of the amino-terminal halves of the NR2 subunits are homologous to AMPA receptor subunits (18). The long carboxyl-terminal halves of the NR2 subunits extend into the cytoplasm (19, 20), forming tails that serve as nucleation sites for the NMDA receptor-associated signaling complex (Fig. 2).

CaMKII. The most abundant signaling protein in the PSD fraction is Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII), which makes up 1 to 2% of the

total protein in the forebrain (21). CaMKII is a target for Ca^{2+} flowing through the NMDA receptor and is necessary for normal synaptic plasticity in pyramidal neurons (22, 23). It is a large dodecameric heteromultimer assembled in stochastic combinations from two closely related catalytic subunits, α and β (24, 25). In the forebrain, the α subunit is about three times as abundant as the β subunit. Assembly of the holoenzyme and binding of CaMKII to the PSD (26–28), the actin cytoskeleton (29), or other subcellular sites (30) are mediated by the carboxyl-terminal association domains of the subunits. The structure of the homomeric α -subunit holoenzyme, determined by cryo-electron microscopy, is a gear-shaped ring approximately 20 nm in diameter and 20 nm in thickness, with sixfold rotational symmetry (six "teeth") (25). Each tooth is formed by an antiparallel dimer of the association domains of two subunits. The symmetry of the central ring suggests that each holoenzyme contains six sets of similar binding sites that can mediate subcellular localization.

The cytosolic tails of the NR2 subunits of the NMDA receptor bind to CaMKII and thus can serve as docking sites for it in the PSD (26, 27, 31). The NR2 subunits are phosphorylated by CaMKII (31), but high-affinity binding is mediated by the association domains of the kinase rather than by the catalytic domains (26, 27). Docking of CaMKII to the tail of the NMDA receptor would position its catalytic domains near the mouth of the receptor, ideally located for activation by Ca^{2+} flowing through the channel. The catalytic domains form two hexagonal rosettes situated on opposite faces of the central ring. Because the two rosettes are separated

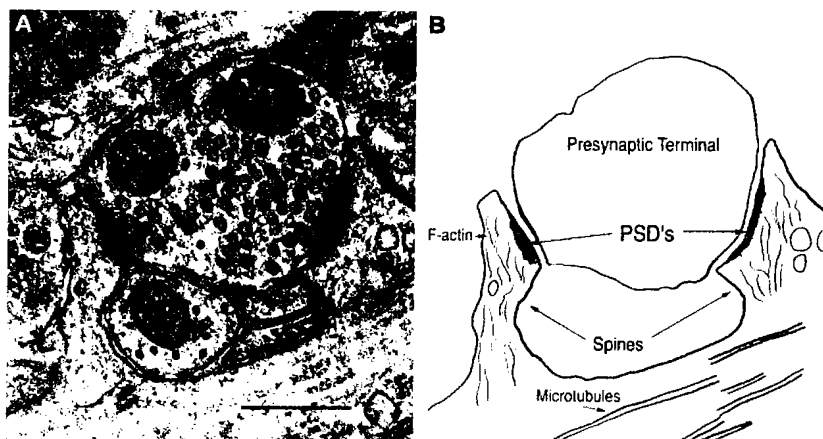


Fig. 1. (A) A presynaptic terminal forming glutamatergic synapses with two dendritic spines. Synaptic vesicles containing glutamate cluster at the site of synaptic contact. (B) Tracing of (A), identifying major synaptic structures. The dendritic shaft contains microtubules along which material is transported from the cell body. Spines contain an actin cytoskeleton that confers movement (73) and permits transport of material toward the PSD at the tip of the spine. Scale bar in (A), 400 nm.

by almost 20 nm (25), each receptor-bound holoenzyme could, in theory, rapidly phosphorylate several nearby substrate proteins, including other signaling molecules in the PSD (32), as well as AMPA receptors, which are up-regulated by phosphorylation by CaMKII after NMDA receptor activation (8, 9).

PSD-95. Another central component of the NMDA receptor signaling complex is the scaffold protein PSD-95 (also referred to as SAP-90). The PSD-95 family comprises four closely related proteins (sometimes called MAGUK proteins), each of which contains five protein-binding domains (15). Three amino-terminal PDZ (PSD-95, Discs-large, ZO-1) domains are followed by an SH3 domain and a GuK domain homologous to yeast guanylate kinase but lacking enzymatic activity. The first and second PDZ domains bind tightly to the tails of the NR2 subunits of the NMDA receptor (33, 34). The three PDZ domains each have slightly different binding specificities and can interact with a variety of different neuronal membrane proteins (35). It remains unclear how interactions with the PDZ domains are regulated during synaptic development and which of their possible interactions predominate in glutamatergic synapses. Nevertheless, the tight colocalization of NMDA receptors and PSD-95 at synapses and the abundance of both proteins in the PSD fraction suggest that in the forebrain,

many synaptic NMDA receptors are attached to the PDZ domains of PSD-95 or one of its family members (15).

At least three other synaptic proteins can interact with the remaining PDZ domains of PSD-95: neuronal nitric oxide synthase (nNOS) (36), neuroligin (37), and SynGAP (32, 38). Of these three, the evidence is strongest that SynGAP is associated with PSD-95 in many glutamatergic synapses on forebrain pyramidal neurons. SynGAP is a synaptic ras GTPase-activating protein (rasGAP), with a GAP domain homologous to that of the canonical rasGAP p120 (39). SynGAP is specifically expressed in neurons and is highly concentrated at synaptic sites in hippocampal neurons, where it is tightly colocalized with PSD-95 (32, 38, 40). SynGAP is almost as abundant in the PSD fraction as PSD-95 itself (32), suggesting that many synaptic PSD-95 molecules are bound to at least one copy of SynGAP.

The function of rasGAPs is to accelerate the intrinsic guanosine triphosphatase (GTPase) activity of ras, thus accelerating the rate of inactivation of the GTP-bound form of ras (39). Because the most common downstream effect of GTP-ras is activation of the MAP kinase (ERK 1 and ERK 2) cascade, rasGAPs can be thought of as brakes on the MAP kinase pathway. How might the function of SynGAP be linked to the NMDA receptor? The rasGAP activity is strongly inhibited by phosphorylation of SynGAP by

CaMKII, an early target of calcium flowing through the NMDA receptor (32). Hence, activation of the NMDA receptor may lead directly to inhibition of SynGAP and release of the brake on the MAP kinase pathway. An important missing link in this scheme is the nature of signaling pathways at glutamatergic synapses that can activate ras. Possible candidates include src or fyn, which can activate ras through the N-Shc adaptor protein (41), or the BDNF (42) and Ephrin/EPH pathways (43). Postulated dendritic targets for regulation by MAP kinase include A-type K^+ channels that modulate the sizes of EPSPs and of back-propagating action potentials (10) and MAP2, which may mediate cellular remodeling (44).

Neuronal NOS, a Ca^{2+} -activated form of NOS, can bind to PSD-95 through a class III PDZ domain interaction in which its own amino-terminal PDZ domain binds to a PDZ domain of PSD-95 (45). Neuronal NOS is not abundant in the PSD fraction and is not expressed at high levels in pyramidal neurons. However, it is highly expressed in certain γ -aminobutyric acid-containing neurons, which also express members of the PSD-95 family (46). Therefore, PSD-95 may concentrate nNOS near the NMDA receptor at postsynaptic sites in these neurons.

Neurologin is an adhesion molecule that is present throughout the soma and dendrites of many neurons and has been localized to the synaptic cleft and the postsynaptic density of some neurons (47). It has not been detected in substantial amounts in the PSD fraction (32, 48); thus, its association with PSD-95 may be transient, or more easily disrupted, than that of other proteins by extraction with detergent during purification of the PSD fraction. Alternatively, it may associate with PSD-95 in a relatively small proportion of synapses. The recent finding that expression of neurologin in heterologous cells can induce clustering of presynaptic vesicles in contacting axons suggests that neurologin may help to induce synapse formation at potential postsynaptic sites that contain NMDA receptor-associated signaling complexes (49).

The Homer/Shank Complex

A third class of glutamate receptors present at many excitatory synapses is the metabotropic or heterotrimeric GTP-binding protein-linked glutamate receptors (mGluRs). Subtypes mGluR1 and mGluR5 are concentrated around the outer rim of glutamatergic PSDs as well as in decreasing concentration in the spine membrane as a function of distance from the PSD (50). Their activation by glutamate leads to production of inositol trisphosphate and the consequent release of calcium through the IP_3 receptor from internal membrane stores into the cytosol. Many spines contain membrane vesicles that store

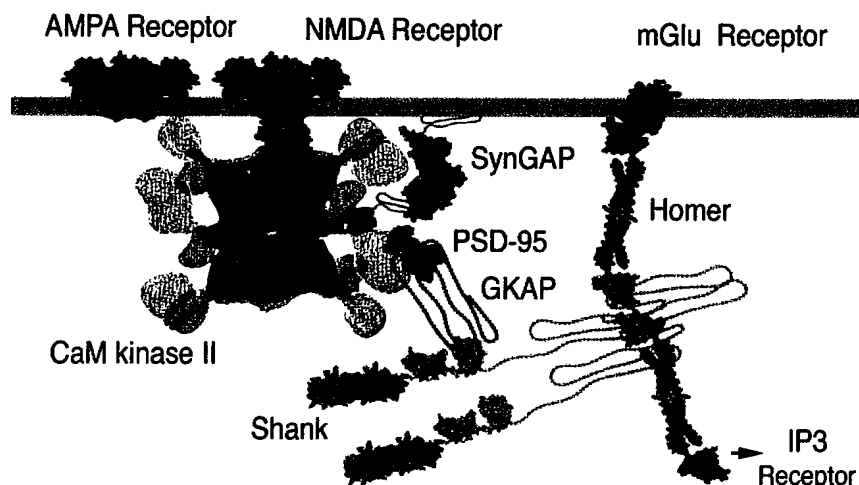


Fig. 2. Cartoon of two hypothetical signaling machines in the PSD. One comprises the NMDA receptor, CaMKII, PSD-95, and SynGAP. The other links the mGluR to the IP_3 receptor and to the NMDA receptor complex via a scaffold assembled from Homer and Shank. The structures of these synaptic proteins have not been determined in their entirety; however, structures of several homologous domains are in the GenBank database and have been used here to portray the relative sizes and positions of contact among the proteins (74). Where no homologous structure is available (GKAP, portions of Shank, portions of SynGAP, and the cytosolic tail of the NMDA receptor), proteins are represented by beaded lines or forms depicting their approximate size estimated from their molecular weight. The structure of the CaMKII holoenzyme was determined to ~30 Å resolution by cryo-electron microscopy (25). The holoenzyme is positioned behind the NMDA receptor and PSD-95 for clarity. It would extend approximately 20 nm into the space behind the plane of the diagram. The individual catalytic domains are depicted as stippled structures extending from the central core.

calcium (these vesicles are sometimes called the spine apparatus). Recent work suggests that a lattice of scaffold proteins may link the cytoplasmic face of mGluRs to IP₃ receptors in the spine apparatus. The lattice may also be connected to PSD-95 and thus to the NMDA receptor complex.

The hypothetical link is based on the properties of the scaffold protein Homer, which contains an amino-terminal EVH1 domain followed by a rod-shaped coiled-coil domain (57). The EVH1 domain can bind the cytosolic face of mGluRs and also the IP₃ receptor. Because Homer dimerizes via the coiled-coil domains to form a rod with EVH1 domains on either end, it could link the two receptors together. The linkage formed by one Homer dimer would be short; however, the EVH1 domain can also bind a specific site on Shank, a ~170-kD protein with several protein binding domains (52) (Fig. 2). One of these domains binds a protein termed GKAP (guanylate kinase-associated protein) that in turn binds to the Guk domain of PSD-95. Because Shank can associate with itself, forming homomultimers, it is postulated to act as a backbone, permitting the formation of a three-dimensional network of Shank multimers and Homer dimers, linking together the NMDA receptor complex in the PSD, mGluRs in the membrane adjacent to the PSD, and the IP₃ receptor located on intracellular calcium storage vesicles (53) (Fig. 2).

Other Signaling Complexes

Biochemical and pharmacological evidence indicates that a number of other signaling complexes are located in spines within or near the PSD. For example, AMPA receptors may be bound to their own unique set of signaling complexes (54). In addition, the cyclic adenosine monophosphate (cAMP) signaling pathway is implicated in the regulation of glutamatergic transmission. cAMP potentiates induction of long-term potentiation (LTP) in the Schaeffer collateral pathway (55). The favored mechanism involves a regulatory cycle first postulated in liver and muscle (56) and now well documented in dopaminergic transmission (57). Activated cAMP-dependent protein kinase phosphorylates a protein called Inhibitor-1 (DARPP-32 in the dopaminergic pathway). Upon phosphorylation, Inhibitor-1 becomes an inhibitor of protein phosphatase-1. This inhibition potentiates phosphorylation of proteins that can be dephosphorylated by protein phosphatase-1. In Schaeffer collateral synapses, the cAMP pathway "gates" autophosphorylation of CaMKII and subsequent induction of LTP (55).

Phosphatase-1 and the cAMP-dependent protein kinase can be complexed with the NR1 subunit of the NMDA receptor by the scaffold protein Yotiao (58, 59), a splice variant of a family of AKAP (A-kinase-associated protein) proteins that target the cAMP-dependent protein kinase to subcellu-

lar compartments (60). Yotiao and a second AKAP, AKAP75/150, which targets protein kinase A, protein kinase C, and the Ca²⁺-dependent protein phosphatase calcineurin to dendritic microtubules, can be detected by immunoblot in the PSD fraction and in immunoprecipitates of the NMDA receptor (58, 59, 61–63). Their relatively low abundance in the PSD fraction suggests that they may be present in a subset of PSDs in the brain. Differential distribution of AKAPs could alter the forms of synaptic plasticity displayed by different synapses.

Pharmacological evidence also indicates that protein kinase C (64, 65) and the MAP kinase pathway (66, 67) participate in postsynaptic regulation of synaptic plasticity at glutamatergic synapses. The structural basis for their localization in spines is not yet firmly established.

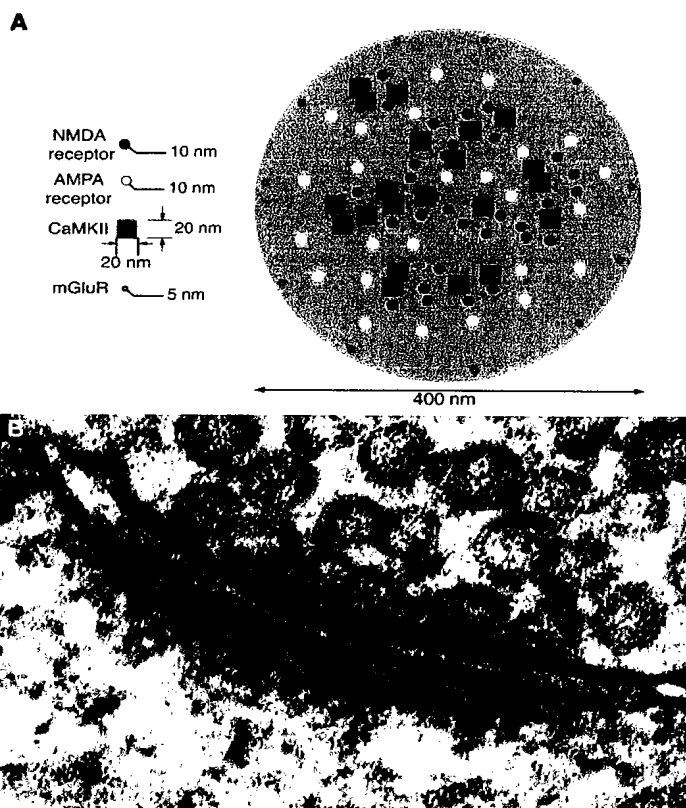
Just as for protein kinases, appropriate location and regulation of protein phosphatases are crucial for proper metabolic control. The calcium-dependent protein phosphatase calcineurin is localized in dendritic spines (68), perhaps by AKAP75/150 (60). Both Yotiao (58) and the neurabin/spinophilin family of proteins (69) could target protein phosphatase-1 to dendritic spines where it can dephosphorylate a variety of substrates. The ubiquitous protein

phosphatase 2A is regulated and targeted by tissue-specific subunits; its brain-specific regulatory subunits have just begun to be studied (70).

Conclusions

Progress in understanding the biochemical and structural basis of synaptic regulation has been rapid and exciting over the past few years. It has been fueled by the recognition among cell biologists that signaling specificity results from the formation of protein complexes that respond locally and discretely to signals from the membrane surface. A few synaptic signaling "machines" have now been identified, but many more remain to be characterized before we unravel the intricacies of signal processing in the brain (Fig. 3). We face two major challenges. The first is to understand to what extent the presence and organization of signaling machinery varies among different synaptic types. This information is crucial because the complement of signaling complexes at a synapse determines the rules by which it integrates and encodes information. The second challenge is to understand how the different signaling pathways interact with and feed back on each other to maintain homeostasis while processing, integrating, and storing rapidly changing information. Efforts to meet this challenge will be aided by promising new strategies for creating and

Fig. 3. (A) Hypothetical scaled diagram of arrangements of NMDA (blue), AMPA (beige), and mGlu (orange) receptors and of CaMKII (red) in a 400-nm-diameter PSD. The diameters of NMDA and AMPA receptors are ~10 nm, and the diameter of the mGluR is ~5 nm. Estimates of numbers of NMDA receptors vary, but average around 50 for a 400-nm PSD (75–79). AMPA receptors cycle in and out of the postsynaptic site, and their numbers are believed to vary from none (a silent synapse) to around 50. **(B)** Protein complexes from Fig. 2 are overlaid to scale on the 400-nm-diameter PSD from Fig. 1A. The diameter of a synaptic vesicle is ~40 nm, and the thickness of an electron microscopy section is ~60 nm. The receptors and CaMKII fit easily into the area of the PSD, suggesting that additional proteins are likely to be present in the PSD in vivo.



testing spatially accurate computer simulations of complex biochemical signaling machinery (71, 72). We have come a long way toward understanding how synapses work, but we still have far to go.

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REVIEW

Actin-Based Plasticity in Dendritic Spines

Andrew Matus

The central nervous system functions primarily to convert patterns of activity in sensory receptors into patterns of muscle activity that constitute appropriate behavior. At the anatomical level this requires two complementary processes: a set of genetically encoded rules for building the basic network of connections, and a mechanism for subsequently fine tuning these connections on the basis of experience. Identifying the locus and mechanism of these structural changes has long been among neurobiology's major objectives. Evidence has accumulated implicating a particular class of contacts, excitatory synapses made onto dendritic spines, as the sites where connective plasticity occurs. New developments in light microscopy allow changes in spine morphology to be directly visualized in living neurons and suggest that a common mechanism, based on dynamic actin filaments, is involved in both the formation of dendritic spines during development and their structural plasticity at mature synapses.

Dendritic spines are the contact sites for most excitatory synapses in the brain (1, 2) where they occur in vast numbers, estimated to be

on the order of 10^{14} for the human cerebral cortex. Spines are particularly associated with neurons where inputs from diverse

sources converge, such as pyramidal cells in the cerebral cortex, whose dendrites commonly have several thousand spines, each representing an excitatory synapse (3–6) (Fig. 1, A and B). Characteristically, spine morphology consists of an expanded head connected to the dendrite shaft by a narrower neck (Fig. 1, C and D), but “stubby” spines lack the neck, whereas filopodia-like “headless” spines also occur, especially during development (4, 7–9). This distinctive architecture depends on a specialized underlying structure of cytoskeletal filaments. In contrast to the dendritic shaft, whose cytoplasm is

Friedrich Miescher Institute, Maulbeerstrasse 66, 4058 Basel, Switzerland.

E-mail: matus@fmi.ch